

Scanning Electron Microscopy of Antennal Structures of Five *Haematopinus* (Anoplura: Haematopinidae)

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Abstract: Scanning electron microscopy is used to demonstrate the possible use of antennae of Anoplura for taxonomic purposes. Of the five *Haematopinus* studied there are sensilla coeloconica, one on the distal posterior aspect of the fourth, and one on the posterior aspect of the fifth antennal segments. There are two pore organs that appear associated with the sensilla coeloconica of the fifth segment. The sizes of the atria of the sensilla coeloconica and the location and distinctness of the pore organs are found to vary considerably and thus may be of taxonomic significance.

Scanning electron microscopy has been used by Miller (1969, 1970 a, b) Hinton et al. (1969) and Hayes et al. (1967) for studies of the surface of insects at both low and high magnifications to give greater depth of field than possible with light microscopy. Specimen preparation is easy and results in little or no demonstrable distortion of surface morphology. Thus minute surface organs of insects may be studied. Such detailed studies may lead to better understanding of phylogeny and physiology of these organisms.

The procedure for studying representatives of the Order Anoplura requires clearing of the specimens in a 10% solution of caustic potash (KOH) until the soft body contents are completely liquified. While rendering the sclerotized plates and genitalia more distinct, this usually causes collapse and distortion of the membranes and other less sclerotized areas. The dissolving technique is often considered the most effective method for lice (Hopkins, 1949). The procedure enables the recovery of the total parasite fauna. However, the resulting specimens are of little value for study of the minute surface details with the Scanning Electron Microscope (S.E.M.). Thus, for studies with the S.E.M. either brushing or searching techniques are required.

Studies with the S.E.M. are limited to the surface structures since it is not capable of penetrating the thinnest membrane. Although topographical views of ultra-

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structure are shown in greater detail the genitalia are not as visible. This may lead to difficulties in classification if observations are limited to the S.E.M.

One of the problems in working with the Anoplura is the lack of available material. For studies with the S.E.M. it is practical to develop taxonomic procedures using surface structures instead of the internal organs. The antennae and their sensilla are here proposed to supplement the internal structures currently used in taxonomy.

Miller (1969, 1970 a, b) using the S.E.M. demonstrated that the antennal sensilla, particularly those of the fourth and fifth or terminal segments, vary considerably among the families of Anoplura studied. The present S.E.M. study of five species of *Haematopinus* was made to determine whether or not variations in antennal sensilla and their integument is evident among species.

MATERIALS AND METHODS

One hundred-twenty specimens were examined! Twelve specimens of *Haematopinus asini* (Linn.) furnished by W. L. Jellison of Parke-Davis Memorial Library, Hamilton, Montana; 12 specimens of *H. asini* furnished by J. S. Dunlap of Washington State University; 12 specimens of *H. eurysternus* (Nitzsch), furnished by Ke Chung Kim of Pennsylvania State University; 24 specimens of *H. eurysternus* (Nitzsch), furnished by W. P. Meleney of U.S.D.A., Albuquerque, New Mexico; 24 specimens of *H. quadripertusus* Fahrenholz also furnished by W. P. Meleney; 12 specimens of *H. suis* (Linn.) furnished by J. S. Dunlap; 12 specimens of *H. suis* (Linn.) furnished by E. M. Nevill of the Department of Agricultural Technical Services, Republic of South Africa; 12 specimens of *H. tuberculatus* (Burmeister) furnished by R. E. Lewis of Iowa State University.

Six adult males and six females were examined from each lot except for *H. eurysternus* furnished by W. P. Meleney, where 12 nymphal stages were also examined, and with *H. quadripertusus* where four males, sixteen females and four nymphs were examined. The *H. suis* furnished by E. M. Nevill were cleared specimens upon receipt. All the specimens were received in either 70% alcohol or alcohol/glycerin. They were washed in fresh 70% ethyl alcohol prior to being mounted. The specimens were cemented to the specimen stubs using aluminum paint. Four specimens of each sex from each lot were cemented dorsal side up and the remaining specimens were cemented ventral side up. The nymphs were cemented dorsal side up except for two from each lot which were placed ventral side up. They were then air dried and coated with approximately 200 angstroms of gold in a vacuum evaporation chamber and studied with a Cambridge Steroscan Mark II S.E.M. at 20 kV. Each louse was completely examined by S.E.M. prior to the studies of the antennal structures. The specimens are on file at the Parasitology Section, Department of Pathology and Laboratories of Nassau County Medical Center, East Meadow, New York.

HAEMATOPINUS ASINI (LINN.)

The morphological characteristics of the fourth and fifth antennal segments of the 12 specimens studied are similar. The opening of the sensillum coeloconicum of the fourth antennal segment is located near the distal apex of the segment on the posterior aspect. This opening is slightly oval and measures approximately 4 by 6 microns. It is surrounded by 4 to 6 concentric rings that appear to be formed by the cuticle of the segment (Fig. 1). The opening of the sensillum coeloconicum of the fifth or terminal antennal segment is located slightly posterior to the center of the segment on its posterior aspect. This opening measures 4 to 5 microns in diameter. There are two pore organs located anteriorly and on opposite sides of the sensillum (Fig. 2). These pore organs each measure approximately 4 to 5 microns in diameter. There are numerous concentric rings (5 to 7) originating from around the sensillum. There are no separate rings around the individual pore organs, thus the organs are not distinct.

The entire antenna appears strongly sclerotized and smooth except for the fifth segment. The texture of this segment particularly that distal to the sensillum coeloconicum is slightly squamate.

HAEMATOPINUS EURYSTERNUS (NITZSCH)

The morphological characteristics of the fourth and fifth antennal segments of the 24 adults studied are similar. The 12 nymphs examined exhibited fusing of the two terminal segments, leading to an elongated fourth terminal segment. However, the sensilla coeloconica, pore organs, and setae appear in the proximal areas where they normally occur in the adult stages, the texture of the segment, although not as strongly sclerotized as in the adults, is similar to them.

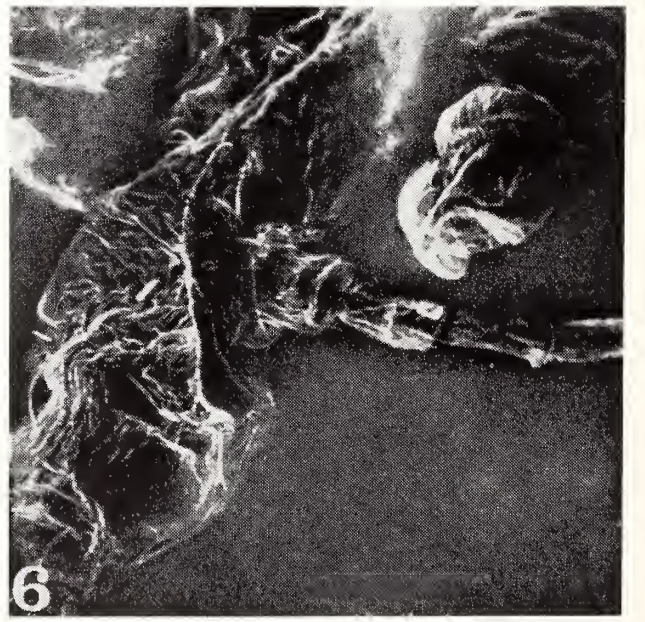
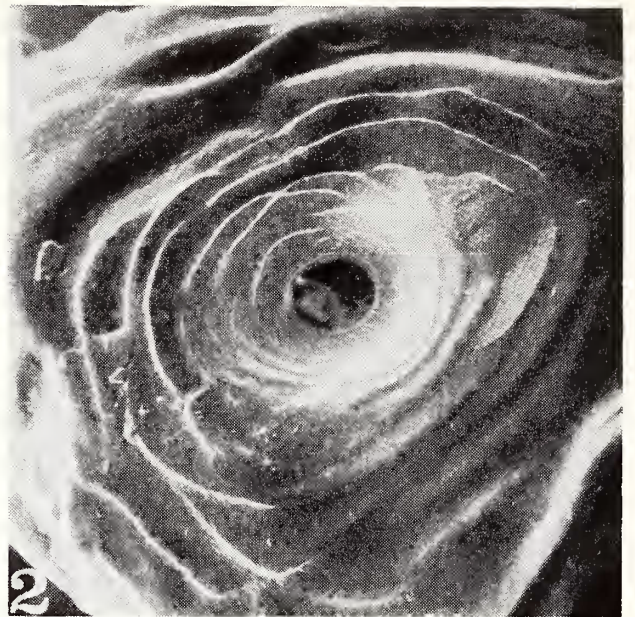
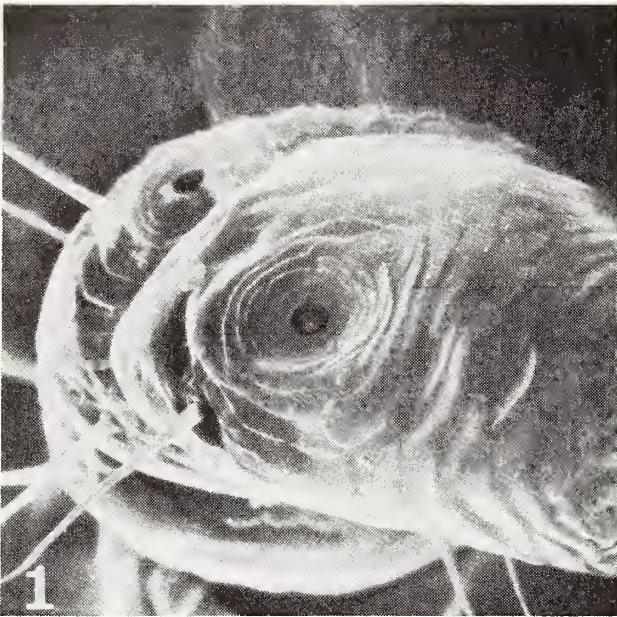
The opening of the sensillum coeloconicum of the fourth antennal segment is located near the distal apex of the segment on the posterior aspect. This opening is slightly oval and measures approximately 4 by 6 microns. It is surrounded by concentric rings and is relatively indistinguishable from that found on the fourth antennal segment of *H. asini* previously described.

The opening of the sensillum coeloconicum on the fifth or terminal antennal segment is located on the proximal quarter of the segment. The opening measures approximately 6 to 7 microns in diameter. It is surrounded by several (3-5) concentric rings that appear to be formed by the cuticle of the segment. Just anterior to the sensilla are two pore organs (Figs. 3-4) which each measure approximately 6 microns in diameter. Their two pore organs are very distinct and each is surrounded by its own concentric rings. There is a fusion of these rings where they meet giving the appearance of a plate. The integument of the antennae is generally smooth except for the rudimentary ridges noted distal to the sensilla coeloconica.

HAEMATOPINUS QUADRIPERTUSUS (FAHRENHOLZ)

The morphological characteristics of the fourth and fifth antennal segments of the 20 adults studied are similar. The 4 nymphs examined showed fusion of the two terminal segments, leading to an elongated fourth terminal segment. However, the sensilla coeloconica, pore organs, and setae appear in the proximal areas where they normally occur in the adult stages. The texture of the segment although not as strongly sclerotized as that of the adults is similar.

The opening of the sensillum coeloconicum of the fourth antennal segment is located near the distal apex of the segment of the posterior aspect. This opening is slightly oval



and measures approximately 3 by 5 microns. It is surrounded by concentric rings and is relatively indistinguishable from those previously described.

The opening of the sensillum coeloconicum on the fifth or terminal antennal segment is located on the proximal quarter of the segment (Fig. 5). The opening measures approximately 3 to 4 microns in diameter. It is surrounded by several concentric rings that appear to be formed by the cuticle of the segment. Just anterior to the sensilla are two pore organs which each measure approximately 6 microns in diameter. The two pore organs are very distinct and each is surrounded by its own concentric rings. There are rings common to both pore organs and the sensilla coeloconicum, however, no plate effect is evident. The integument of the antennae is generally smooth except for the rings around the sensillum coeloconicum and pore organs.

HAEMATOPINUS SUIS (LINN.)

The morphological characteristics of the fourth and fifth antennal segments of the 12 cleared specimens varied considerably. This variation is primarily in the degree of collapsing and folding of the integument. This distortion is best shown by comparing the head of the cleared specimen (Fig. 6) with a similar view of an uncleared specimen (Fig. 7).

The morphological characteristics of the fourth and fifth antennal segments of the 12 uncleared adults studies are similar. The opening of the sensillum coeloconicum of the fourth antennal segment is located near the distal apex of the segment on the posterior aspect. This opening measures 1.5 to 2.5 microns in diameter (Fig. 8). It is surrounded by concentric rings faintly outlined in the cuticle.

The opening of the sensillum coeloconicum of the fifth or terminal antennal segment is located close to the center of the segment on the posterior aspect. The opening measures approximately 1.5 to 2.5 microns in diameter. It is surrounded by several concentric rings. The integument of the antennae is generally smooth except for the rings around the sensilla coeloconica and pore organs. There are faint lines evident on the fifth segment distal the pore organs.

HAEMATOPINUS TUBERCULATUS (BURMEISTER)

The morphological characteristics of the fourth and fifth antennal segment of the 12 specimens studied are similar. The opening of the sensillum coeloconicum of the fourth antennal segment is located near the distal apex of the segment on the posterior aspect. This opening is slightly oval and measures approximately 4 by 6 microns. It is surrounded by concentric rings of the cuticle.

The opening of the sensillum coeloconium of the fifth of terminal antennal segment is

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FIG. 1. Sensilla coeloconica of fourth and fifth antennal segments of *Haematopinus asini*. 1,000×.

FIG. 2. Sensillum coeloconicum and pore organs of fifth antennal segments of *H. asini*. 2,200×.

FIG. 3. Sensillum coeloconicum and pore organs on fourth and fifth antennal segments of *H. eurysternus*. 1,000×.

FIG. 4. Pore organ on fifth antennal segment of *H. eurysternus*. 5,500×.

FIG. 5. Sensillum coeloconicum and pore organs on fifth antennal segment of *H. quadripertusus*. 1,000×.

FIG. 6. Head and antenna of female *H. suis* that had been cleared prior to examination. 160×.

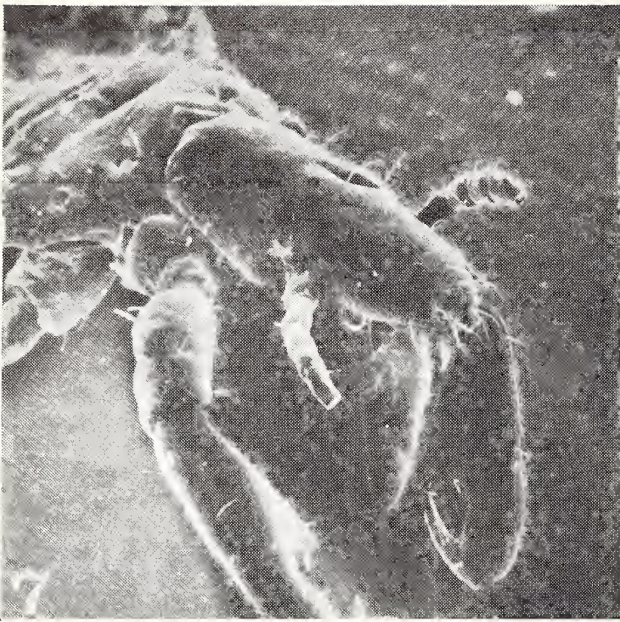


FIG. 7. Head and antenna of female *H. suis* uncleared. 50 \times .

FIG. 8. Sensillum coeloconicum of fifth antennal segment of *H. suis*. 2,000 \times .

FIG. 9. Sensillum coeloconicum and pore organs on fifth antennal segment of *H. tuberculatus*. 2,000 \times .

FIG. 10. Paratergal and tergal plates and genital plates seen on segments 3 through 9 of *H. eurysternus*. 80 \times .

located on the proximal quarter of the segment (Fig. 9). This opening measures 3 to 4 microns in diameter. Just anterior to the sensillum are two organs which each measure approximately 6 microns in diameter. The two pore organs are surrounded by their own concentric rings, rings common to both, and also rings common to the sensillum coeloconium. However, the pore organs are not as distinct as seen in *H. eurysternus*. The integument of the antennae is generally smooth except for the fifth segment where starting at the region of the sensillum coeloconicum and extending to the apex of the segment there are numerous ridges and a somewhat squamous appearance is noted.

TABLE 1

| Species of <i>Haematopinus</i> | Sensilla coeloconica | | Pore Organs Size Microns | Location in relation to Sensilla coeloconica & distinctness |
|-----------------------------------|---|---|-----------------------------|---|
| | 4th Antennal Segment Sizes in Microns | 5th Antennal Segment Sizes in Microns | | |
| <i>H. asini</i> | 4 by 6 oval | 4-5 | 4-5 | on sides of indistinct |
| <i>H. eurysternus</i> | 4 by 6 oval | 6-7 | 6 | anterior to distinct |
| <i>H. quadripertusus</i> | 3-5 round | 3-4 | 6 | anterior distinct |
| <i>H. suis</i> | 1.5-2.5 round | 1.5-2.5 | 5-6 | anterior distinct |
| <i>H. tuberculatus</i> | 4 by 6 oval | 3-4 | 6 | anterior somewhat indistinct |

DISCUSSION

Differentiation between *Haematopinus quadripertusus* and *H. eurysternus* has been demonstrated by using the S.E.M. Ferris (1951) states that although *H. quadripertusus* from cattle in tropical countries is generally much larger, no morphological differences could be detected between it and *H. eurysternus*. However, Stojanovich and Pratt (1966) in their pictorial keys differentiate the two as distinct species. Separation is based upon the more acute and longer median projections of the thoracic sternal plate of *H. quadripertusus* as well as the presence of only four setae on the male genital plate as opposed to the six found on *H. eurysternus*. These differences were also shown by W. P. Meleney (1969). Examination of the antennae indicated differences in the sizes of the openings of the sensilla coeloconica and the texture of the integument in the areas or region of the pore organs, indicating that they represent two distinct species.

Examination of the antennae of the five species revealed differences (Table 1) in the size of the openings, location and variation in distinctness of the pore organ, and differences in the texture of the integument of the distal portion of the fifth or terminal segment. By combining these features with the size and shape of various plates and number of setae it should be possible to determine the species of Anoplura without use of the internal structures. The antennal morphological characteristics of the adults were also present in the nymph stages examined. Previous studies (Miller 1970a) showed that even when there is sexual dimorphism of the antennae that the number of setae, sizes of pore organs and openings of the sensilla coeloconica did not seem to vary. Studies are continuing in an attempt to develop taxonomic keys for the Anoplura based on the antennal characteristics and surface morphology using the S.E.M.

Literature Cited

- FERRIS, G. F. 1951. The Sucking Lice. Pacific Coast Entomol. Soc. 320 pp., illus.
- HAYES, T. L., R. F. W. PEASE, AND A. S. CAMP. 1967. Steroscopic Scanning Electron Microscopy of Living *Tribolium confusum*. J. Insect Physiol, **13**: 1143-1145.
- HINTON, H. E., AND R. SILBERGIED. 1969. Stridulatory Files as Diffraction Grating in Mutillid Wasps. J. Insect Physiol., **15**: 549-552.
- HOPKINS, G. H. E. 1949. Host-associations of the lice of mammals. Proc. Zool. Soc. Lond. **119**: Part II.
- MELENEY, W. P. 1969. A Comparison of *Haematopinus eurysternus* from New Mexico and *H. quadripertusus* from Puerto Rico. Paper presented at 44th Annual Meeting Amer. Soc. of Parasitologists.
- MILLER, F. H. 1969. Antennal Tuft Organs of *Pediculus humanus* Linn. and *Phthirus pubis* (Linn). J. N.Y. Entomol. Soc. **77**: 85-89.
- . 1970a. Scanning Electron Microscopy of Some of the Antennal Structures of *Polyplax serrata* (Burmeister). J. N.Y. Entomol. Soc. **78**: 33-37.
- . 1970b. Scanning Electron Microscopy of *Solenopotus capillatus* Enderlein. J. N.Y. Entomol. Soc. In Press.
- STOJANOVICH AND PRATT. 1966. Pictorial Keys-Arthropods, Reptiles, Birds and Mammals of Public Health Significance. P.H.S. Publication No. 1955 Communicable Disease Center, Atlanta, Georgia.